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II. IMPLICATIONS AND EXTENSIONS OF THE SERIAL ENDOSYMBIOSIS THEORY OF THE ORIGIN OF EUKARYOTES

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Summary

In comparison to other theories put forward so far, the Serial Endosymbiosis Theory appears to be the most critically favoured as an explanation for the origin of mitochondria and chloroplasts. For this reason some of its implications are drawn attention to. In particular it is shown that present hierarchical concepts and terminology based on the classical cell theory are inadequate to cope with the S.E.T. (as the theory is abbreviated here). It is shown that the topological relations of mitochondria and chloroplasts to the cell suggest that they are topologically "outside" it, "embraced" rather than lying truly within it. This position is consistent with the S.E.T. On the other hand microtubule-organising-centres are truly within the cell. An autogenous origin for the latter seems to be more likely than the endosymbiotic proposal of Margulis.

In addition to the primary origin of organelles by the transformation of entire prokaryotic endosymbionts, the possibility of the maintenance of eukaryotic organelles produced by one cell, taken into another, is discussed. Examples of some recently discovered, unusual photosynthetic states in ciliates, brought about by temporary foreign chloroplast maintenance, and possibly degeneration of endosymbiotic photosynthetic flagellates, are discussed in this context. The existence of some contemporary examples of photosynthetic bodies which seem to be intermediate between endosymbiotic blue-green algae and chloroplasts, suggests that some organelles may have had a more recent origin than the Precambrian Era, the process being a continuous one.

Some of the difficulties inherent in alternative theories for the origin of eukaryotes are discussed. It is stressed that alternative proposals have not yet received intensive exposition and comparisons with the S.E.T. are thus difficult. Attempts to use single criteria (particularly biochemical) as phylogenetic "markers" should be made with due consideration of the S.E.T.

Finally, it has bearing on the recognition of kingdoms, for it widens the already acknowledged chasm between prokaryotes and eukaryotes, intermediates between the two types of organisation being impossible (as stressed by Margulis).

Introduction

"Those who claim to possess the truth should remember that heterogenesis [spontaneous generation] was once 'the truth'."

ANDRÉ LWOFF (1957)

The origin and control of eukaryotic organelles has been the subject of a great many recent reviews (see references later) and there would seem to be little point in covering much the same material more superficially here. Instead, after some introductory generalities, this contribution will focus on some less-discussed aspects which are hopefully also of general interest. For example, what are the consequences of theories of the origin of organ-

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elles to the concept of biological "levels of organisation"? How does J. D. Robertson's model of cell topology look today, particularly with regard to the endosymbiotic origin of mitochondria and chloroplasts? In addition, the recent discovery of foreign organelles within some marine protists is discussed since it suggests some possible new dimensions to the question of organellar origins, as yet unexplored. These and other observations suggest the possibility that new organelles may be arising secondarily in contemporary organisms. If this is so perhaps there are other organelles of more recent vintage than chloroplasts and mitochondria. After discussing these possibilities briefly the conclusion will contain a personal assessment of the present state of affairs concerning the various theories for organellar origins.

One of the most striking differences between pro- and eukaryotes is the packaging of the genome in the latter into several membrane-bound organelles (Stanier, 1970). In prokaryotes the cytoplasmic membrane (plasmalemma), including any invaginations it may have, is the site of respiratory electron transport and photosynthetic energy processes, whereas these occur isolated within the mitochondria and chloroplasts of eukaryotic cells (Cohen-Bazire, 1971). In prokaryotes the genophore seems to be attached to the membrane, a feature known from the dinoflagellates and radiolaria (in which it is the nuclear membrane) among the eukaryotes. The search for the most plausible explanation for these differences is a major preoccupation for those interested in the evolution of cells.

One can distinguish between two principal schools of thought with regard to eukaryotic organellar origins. The first is the *wholly autogenous* viewpoint. It has been widely held until recently, despite the absence of any detailed exposition. In this view the eukaryotic structure has arisen entirely by the differentiation of the components of one unit ("cell") with increasing compartmentalisation with the passage of time. The isolation of the primary respiratory and photosynthetic machinery within double-membrane-delimitated bags has been assumed to convey a metabolic advantage, although in precisely what manner, other than selective permeability, has not been made clear. Pro- and eukaryotic cells thus represent phylogenetic equivalents (homologues) which have diverged along different lines.

Alternatively there is the *partially xenogenous* view (or *exogenous*, if preferred) in which some organelles, usually the mitochondria and chloroplasts, are thought to have originated from the incorporation and semi-integration of foreign cells between one and two thousand million years ago during the Pre-cambrian Era. Although first suggested near the turn of the century (Schimper, 1883; Altmann, 1890; Mereschkowsky, 1905, 1910, 1920; Famintzin, 1907; and later Wallin, 1927), this theory has only been seriously examined following the demonstration of DNA within chloroplasts (Ris, 1961; Ris & Plaut, 1962) and mitochondria (M. Nass & S. Nass, 1963; S. Nass & M. Nass, 1963).

In 1967 Klein & Cronquist asserted that "this bad penny [the endosymbiotic origin of chloroplasts] has been circulating for a long time," and that "clearly there is no chemical, structural or phylogenetic basis for this belief." Woolhouse (1967) suggested that further speculation along such lines should be abandoned. It is ironic that it was in the same year that Goksøyr (1967) produced an outline, and Margulis published a detailed general hypothesis for the origin of eukaryotic cells by a succession of endosymbioses (under her earlier name of Sagan, 1967). The latter detailed,

provocative and stimulating work has led to the focus of a great deal of comparative ultrastructural, biochemical and genetic work on the question. Proposals regarding the mitochondria and chloroplasts were admittedly not new, and Margulis' spirochete-like prokaryotic origin for the centriole is still doubtful. She suggested the use of mitotic mechanisms as indicators of phylogeny, but had to use figures chiefly derived from light microscopy. It is evident now, however, that ultrastructural information is essential in order to detect significant differences in mitotic mechanisms (eg. Kubai & Ris, 1969; Pickett-Heaps & Marchant, 1972, McDonald, 1972). Another valuable contribution by Margulis was her inclusion of existing geochemical and micropaleontological data in an attempt to provide a time-scale for the postulated events. Principally she deserves recognition for putting together a cohesive, detailed framework for the Serial Endosymbiosis Theory (as the general theory is dubbed here, being the S.E.T. for short), presenting it in its most complete form in a recent book (Margulis, 1970).

Here the essence of the S.E.T. is considered to be the postulate that both mitochondria and chloroplasts have arisen from ancient endosymbioses. It is not restricted to the particular proposals of Goksøyr and Margulis. Authors differ in the sequence in which the events occurred. The above authors believe that the acquisition of the protomitochondrion predated the protochloroplast (as does this author), whereas Stanier (1970) has advocated the reverse. The suggestion that centriole-like structures have also arisen from endosymbiotic cells can be considered an extension to the theory by Margulis, and the coenocytic fusion of prokaryotes to increase the DNA content an extension by Goksøyr. These, and some alternatives will be discussed in the conclusion to this paper.

Before that some less explored aspects of the S.E.T. will be examined, as well as some other possible extensions of it.

Levels of organisation

The sharp disjunction between prokaryotes and eukaryotes is now widely acknowledged, the passing years continuing to re-inforce Stanier & Van Niel's (1962) assertion. Contemporary custom accords the term "cell" to units of both types of organisation, with the added distinction of "non-cellular" or "acellular" categories for some multi-nucleate eukaryotic organisms. "Multicellular" organisms are thought to be the next higher hierarchical level, consisting of multiples of the lower level ("cells"), usually also having an important extra-cellular component binding the cells together. Viruses are thought to exist at a lower level than cells, being "subcellular".

The above is compatible with the wholly autogenous theory of eukaryotic development, for both pro- and eukaryotic cells are thought of as being modifications of the same structural unit, one line having specialised in metabolic diversity while the other underwent internal and external morphological differentiation. It is thus reasonable to use "cell" in both cases, for the units are homologous.

Early in this century Dobell (1911), impressed by the functional equivalence of eukaryotic protists to entire metazoans or metaphytes, hotly disputed the view that the latter were comprised of multiples of the former, objecting to use of the term "unicellular" for protists. Instead he proposed "non-cellular" for use with all eukaryotic protists. In this crusade he found an ally in Hyman (1940) although she preferred the term "acellular".

Their confusion of structural, phylogenetic homology with functional "homology" (if there can be such) was pointed out by Minchin (1912), Baker (1948), Corliss (1957) and others, the application of the electron microscope finally putting the argument to rest by demonstrating the essential structural similarity of all eukaryotic cells. "Acellular" could be applied to bi- or multi-nucleated protists, but this is a problem which is inappropriate to discuss further here.

One of the primary implications of the S.E.T. is that mitochondria and chloroplasts (and perhaps other organelles, but these less likely) have been phylogenetically derived from entire ancestral prokaryotic units. Furthermore, although no longer fully autonomous, they still carry with them information for some of their development. If one accepts the S.E.T. they can be said to be true homologues, in the strictest sense of the word, of present day prokaryotes. Stanier & Van Niel (1962) asserted that prokaryotes are "cells", a view which has not been seriously disputed. This brings us to the odd view of the eukaryotic condition as one in which cell homologues, of one or two fundamentally different types, reside within another larger cell. In other words, the eukaryotic "cell" is a *multiple* of the prokaryotic "cell".

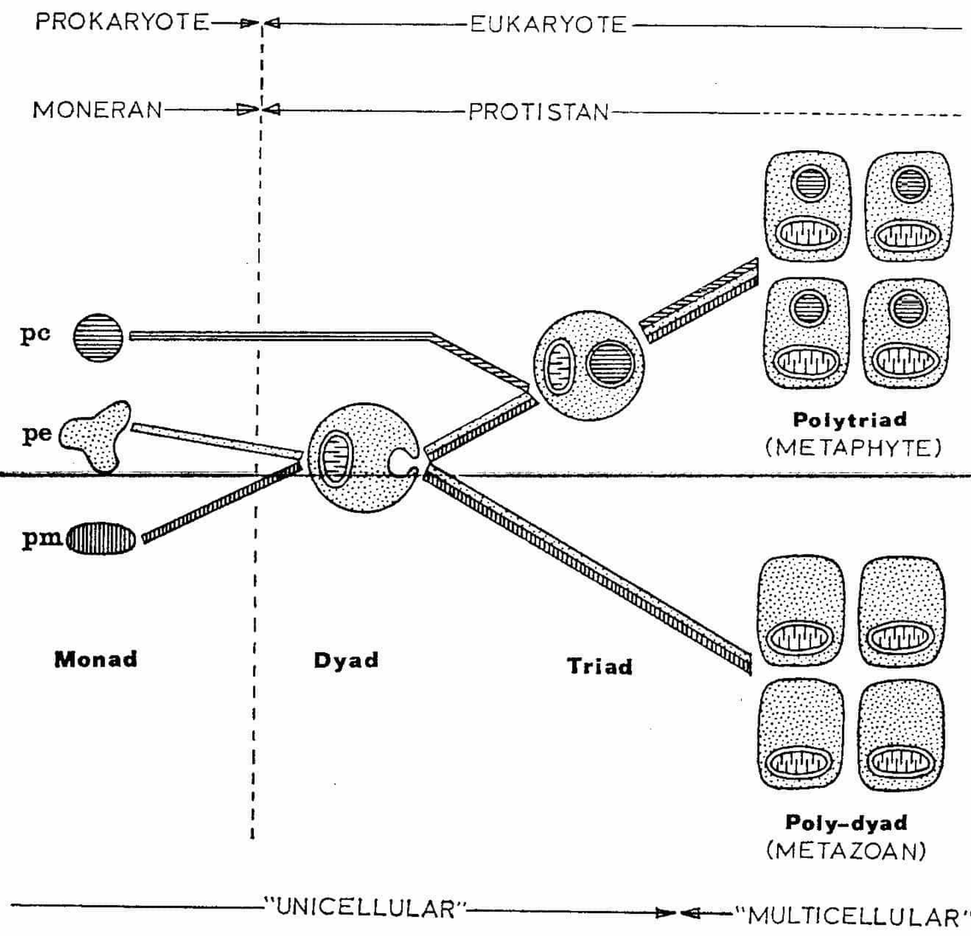


Fig. 1. A diagrammatic representation of the consequences of the Serial Endosymbiosis Theory to the lower levels of organismal organisation (see text for details). Nuclei are not shown. Current terminology is indicated in thin type. Pc, pe, pm represent bacterial ancestors postulated for the chloroplast host and mitochondrion respectively.

Fig. 1 is a diagrammatic representation of this author's views of the implications of the S.E.T. to the lower levels of biological organisation. The coded lines indicate the hypothesised routes of evolution from one level to another (c.f. Margulis, 1970, fig. 2-4). At the left are monogenomic cellular units, termed monads (an old word which seems to have literal value here). Only the three monad types involved in higher levels are shown. Of these there are contemporary prokaryotic equivalents for the protomitochondrion (aerobic eubacteria) and the protochloroplast (Cyanophytes, referred to as "blue-green bacteria" here in the hope that this will further the recognition of their true phyletic affinities). The third type, corresponding to the original host cell, does not seem to have extant representatives. The delimitation of the nucleus in this proto-eukaryote was probably associated with the development of an endoplasmic-reticulum-like system (Robertson, 1962). This does not alter the level of organisation from this point of view, and so this monad may or may not have possessed a membrane-limited nucleus (Margulis, 1970 has provided arguments for the presence of the protomitochondrion prior to the development of the steroid-containing endomembrane system). Multicellular prokaryotes are quite common, particularly among the blue-green bacteria. These palmelloid and filamentous forms, being multiples of essentially the same unit, might be termed "polymonads" (not shown in fig. 1) in coherence with the analytical terminology used here, although, in the view of the S.E.T., they are the only literal multicellular organisms.

The synthesis of proto-eukaryote and protomitochondrion produced a "dyad", consisting of two equivalent units, and it is the dyad which forms the basis for all animal-like eukaryotic stocks. The addition of a protochloroplast to this dyad produced a "triad" which, in turn, is the basic unit for all eukaryotic photosynthetic stocks. Metazoans and metaphytes are then multiples of these (polydyads and polytriads respectively).

The outcome of this, in terms of unitary, cellular make-up, is that the conventional uni- and multicellular terminology is inadequate to cope with the consequences of the S.E.T. Even the distinction of prokaryotic cell, eukaryotic cell, eukaryotic multi-cellular organism, although an improvement, masks the dyad/triad distinction and their "multicellular" products. Schnepf (1966) has proposed the terms protocyte and eucyte for the equivalents of monads and dyads, but he did not take the concept further. His protocyte referred specifically to the protomitochondrion.

The terms suggested here seem to be useful for referring to the different states in analytical discussion providing that they do not mask recognition of the vital element of the integration of components.

Cell topology.

One of the best known models of the eukaryotic cell is that of J. D. Robertson (1962, 1964) which has appeared frequently in basic texts (eg. Swanson, 1969). He proposed that the endoplasmic reticulum, the Golgi apparatus and the nuclear envelope represent differentiated inner portions of the cell surface, its outer expression being the plasmalemma (cell membrane). This was not conceived of as a static plumbing system, but rather as a dynamic network of canals, at times connected, at other times pinching off, conveying the contents of the lacunae both out of and into the heart of the cell. Morré et al. (1971) have recognised the inner parts of this

as the *endomembrane system*, recognising its functional continuity. The view has been supported by numerous electron microscope observations of connections between the above components (cited in Morré et al., 1971). The observation that portions of plasmalemma are synthesised either as parts of the nuclear membrane, or Golgi vesicles, moving outwards through the system, also supports this view (Whaley et al., 1971).

Stanier (1970) has emphasised the considerable importance of this dynamic membrane proliferation and in particular, the associated ability to perform endocytosis (including the intake of objects larger than macromolecules, an ability not shared by contemporary prokaryotes), in providing a selective advantage to the early protoeukaryote. Indeed, it is an essential prerequisite for the development of early endosymbioses. All contemporary endosymbionts, both pro- and eukaryotic, with the exception of some Microsporidia, are isolated from the host cytoplasm by at least two membranes, the outermost of which (essentially a vacuolar membrane) is the means by which the host regulates exchange with the endosymbiont, the inner corresponding to the outermost endosymbiont membrane (usually the plasmalemma). It is intriguing therefore, that both mitochondria and chloroplasts possess a double-membraned envelope. It is tempting to apply a similar interpretation to these outer and inner membranes as that developed for contemporary endosymbioses. How reasonable is such an interpretation?

The outer membranes of both mitochondria and chloroplasts are usually closely and quite regularly spaced from one another (at a distance of approximately 60 A in chloroplasts), a feature which immediately distinguishes them ultrastructurally from endoplasmic reticulum and from the vacuolar membranes involved in endosymbiosis. It is known also that the peri-organellar spaces are not merely fluidic bags, but contain predictable constituents with a fairly high percentage of protein (60 mg per ml of water in the perimitochondrial space: Hackenbrock, 1968). Creatine kinase and adenylate kinase seem to be customarily located within the perimitochondrial space (Hughes et al., 1970). Freeze-etch preparations for electron microscopy have revealed fibrous extensions of the outer membrane in the mitochondrial space (Tewari et al., 1973) and these fibres may impart a certain amount of structural linkage between the outer and inner membranes.

It is possible to separate the mitochondrial envelope membranes experimentally (e.g. Parsons et al., 1967) and, as a result, it has been shown for mitochondria that the outermost is controlled and synthesised by the "host" cell, whereas the inner membrane, bearing most of the respiratory machinery, has components coded for by the mitochondrial DNA (M. Nass, 1971). It has been repeatedly observed that connections between the endoplasmic reticulum and the mitochondrial envelope occur (first by Bernhard & Rouiller, 1956 plus others cited in S. Nass, 1969). Thus, for mitochondria it seems that the outer membrane has structural and biochemical similarities to endoplasmic reticulum, and that the perimitochondrial space can be considered as a topological extension of its lacuna, bearing in mind that it is, however, strongly modified for particular functions.

The situation with chloroplasts is slightly more complicated. Most algal chloroplasts (exceptions being in the red and green algal groups) are known to be wholly or partially surrounded by a specialised form of endoplasmic reticulum, termed the chloroplast endoplasmic reticulum (Bouck, 1965; Gibbs, 1970; Bisalputra, 1973). In some groups the latter is continuous with the nuclear envelope (eg. cryptomonads). This sac of endoplasmic reticulum is, however, exterior to the chloroplast envelope. In dinoflagellates and euglenoids it appears that the outer sac is applied directly to the chloroplast envelope, giving the appearance of a triple membrane surrounding the chloroplast. Despite these secondary elaborations direct contact between the endoplasmic reticulum and the chloroplast envelope has been seen (in the brown alga *Ectocarpus* by Oliviera & Bisalputra, 1973) structural differences between the outer and inner membranes have been demonstrated by Bisalputra & Bailey (1973), and the situation seems to thus be closely analogous to that in mitochondria.[1]

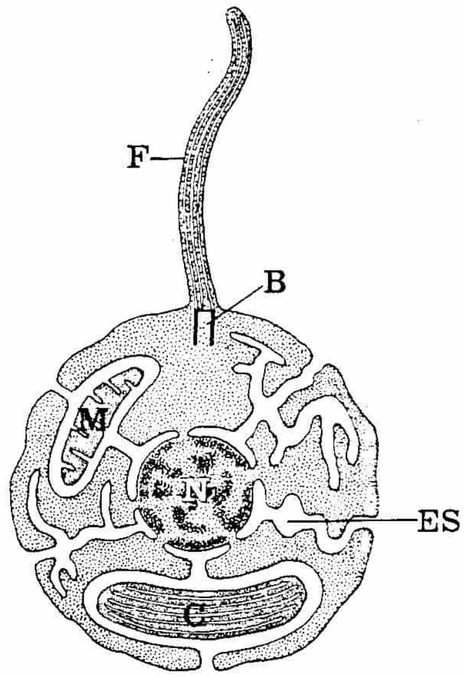


Fig. 2. A topological model of a photosynthetic eukaryotic flagellate, illustrating the possible "outsideness" of the inner mitochondrial and chloroplast components. B: basal body; C: chloroplast; ES: endomembrane system; F: flagellum; M: mitochondrion; N: nucleus.

Topologically speaking, if the lacuna of the endoplasmic reticulum can be considered "outside" the cell then the inner components of mitochondria and chloroplasts are also "outside" the cell. Fig. 2 presents a simplified Robertsonian model of the cell in which the postulated topological relationships of mitochondria and chloroplasts are included. While this does not add much weight to the S.E.T., it is highly compatible with it. In this sense the protomitochondrion and protochloroplast have never truly entered the host cell, but are instead "embraced" by it, a situation which has remained unchanged over the ages even though the membranes surrounding them have become specialised for their tasks of transport and control.

Schnepf (1966) approached this aspect from a slightly different angle.

1. Crotty & Ledbetter (1973) have observed continuities between the endoplasmic reticular membranes, the outer chloroplast membrane, the outer mitochondrial membrane, the plasmalemma, the outer nuclear membrane, the tonoplast, dictyosome and microbody in the fern *Pteris*, providing further support for the interconnected endomembrane concept.

Instead of considering surfaces he used the concept of confluent intermingling of ground-substances (cytoplasm, nucleoplasm, etc.) to determine topological relationships. For example, in most cells the nucleoplasm and cytoplasm intermingle when the nuclear membrane breaks down during nuclear division. Thus they belong to the same "phase" and are topologically similar. Similarly one can recognise the inter-relatedness of those components now termed the endomembrane system (see earlier). This approach points up two snags in the view of the topological "outsideness" (and consequent independent, organismal-like integrity) of mitochondria and chloroplasts. One is the well-known structural reduction of mitochondria in yeasts when exposed to anaerobic conditions, followed by reformation when returned to aerobic conditions (reviewed by Roodyn & Wilkie, 1968). At such times, even though parts of the mitochondria are thought to remain together, the mitochondrial matrix may mix with the general cytoplasm. If so, the mitochondria of yeasts can alternate between being topologically isolated from, and fully integrated with, the host cell.

D. Taylor (1969) has provided an electron micrograph in which the nucleoplasm of the dinoflagellate, *Symbiodinium microadriaticum,* is apparently confluent with cytoplasm associated with an adjacent chloroplast via canals created by endoplasmic reticulum. If this were continuity between the chloroplast stroma and the nucleoplasm it would be difficult to explain in terms of the customary separation of function between the two organelles. However in this micrograph it is not possible to determine which membrane-pair is the chloroplast envelope and it is more probable that the confluence is with cytoplasm outside the chloroplast envelope.

The centriole/basal body (and other microtubule-organising-centres) is inside the host cell, lying freely in the cytoplasm. If it originated as an endosymbiotic cell (as proposed by Margulis), then it became rid of all outer membranes, both of host and symbiont, an apparently unique case. This seemingly trivial feature may be an argument against the independent cellular origin of the centriole, favouring instead either an endogenous origin or a viral-like origin (see further details at the end of the discussion).

Intra-mitochondrial bacteria

Stanier (1970) has stressed another peculiarity of contemporary prokaryotes: their inability to harbour endosymbionts. From the S.E.T. one might imagine that this property has been perpetuated in mitochondria and chloroplasts. Consequently reports of bacteria living within mitochondria require examination in this regard. At least two reports are known to this author, both occurring within ciliates.

The first is by Yamataka & Hayashi (1970), who observed them in *Halteria geleiana,* both in the free-swimming state and when the ciliate was encysted. It is difficult to assess their micrographs, but there seems to be no doubt that rod-shaped, multiple-membranebound bodies 0.4 to 0.5 μm in diameter were present within the mitochondrial matrix. They found 90 or more within one mitochondrion in one instance. Although it is difficult to be certain, the bacteria observed by de Puytorac & Grain (1972) in *Urotricha ovata* appear to be located in the perimitochondrial space and not in the mitochondrial matrix. From the topological point of view outlined above the latter can be considered not truly "inside" the mitochondrion, whereas those of Yamataka & Hayashi (1970) are.

Foreign organellar retention

The S.E.T. has so far been entirely concerned with the primary origin of organelles in eukaryotes. However, one may ask whether it is possible for one eukaryotic species to obtain and maintain an organelle from another species. Studies directed toward determining the degree of independence of chloroplasts and mitochondria have direct bearing on the question, even though not thought of in quite that way. Of particular interest are those involving foreign organellar "*in vivo*" techniques.

M. Nass (1969a) discovered that mouse fibroblasts (L-cells) in tissue culture were able to phagocytose isolated chloroplasts from African Violet and spinach plants and retained them, undigested, at least five days. They also retained foreign mitochondria from chicken liver tissue. Not only did the organelles exhibit a fair degree of structural integrity but the chloroplasts also retained some functional abilities, fixing CO_2 and possessing light reactivity (Hill reaction) when re-isolated from the fibroblast two days after their introduction.

In another experimental approach Giles & Sarafis (1971, 1972) have maintained chloroplasts from the coenocytic green alga *Caulerpa sedoides* in the whites of hens' eggs for periods of up to 21 days. Not only have the chloroplasts remained structurally organised, but Hill reaction activity remains high for the first 108 hours, dropping to one-eighth of the initial value at the end of the period. Chloroplast division has been claimed to occur frequently.

The best known natural counterpart to these experiments is the maintenance of chloroplasts from other coenocytic green algae by "sea slugs": sacoglossan opisthobranch molluscs. This was first observed by Kawaguti & Yamasu (1965) who discovered that the green colouration of the upper surface of the animals was due to the presence of large numbers of chloroplasts occurring within cells of the digestive gland. Since then this phenomenon has been found in other members of the order and it may be present in the majority (Taylor, 1967, 1968, 1970, 1971; Greene, 1970*a*). The chloroplasts can remain in the animals for up to three months (Hinde & Smith, 1972), during which time there is a translocation of appreciable amounts of photosynthate to the tissues of the mollusc (Trench, 1969; Trench et al., 1969; Greene, 1970*b*). It appears that some of the photosynthate can be used as a substrate for mucus synthesis in the animal's pedal gland (Trench et al., 1969). The net photosynthetic rate in *Elysia viridis* has been found to be approximately 40% of that found in the intact alga *Codium fragile* from which the chloroplasts were derived by the animal (Trench et al., in Hinde & Smith, 1972). There are qualitative differences in the function of the chloroplasts within the animal and in the alga. In the former Greene & Muscatine (1972) found that the chloroplasts were unable to synthesize lipids or sucrose. *Elysia viridis* shows light-oriented behaviour (Nicol, 1960) which may be related to its photosynthetic processes.

It is not likely that these examples have direct evolutionary consequences. Photosynthetic mice or chickens do not seem to be in the immediate offing. The sea slugs are the apparent beneficiaries of an inability to digest the chloroplasts in their food, plus a means of subverting their own immune response. It is interesting to note that other close relatives (the aeoliid nudibranchs) can maintain the stinging cells (nematocysts) from their coelenterate food, placing them in dorsal projections for their own defence.

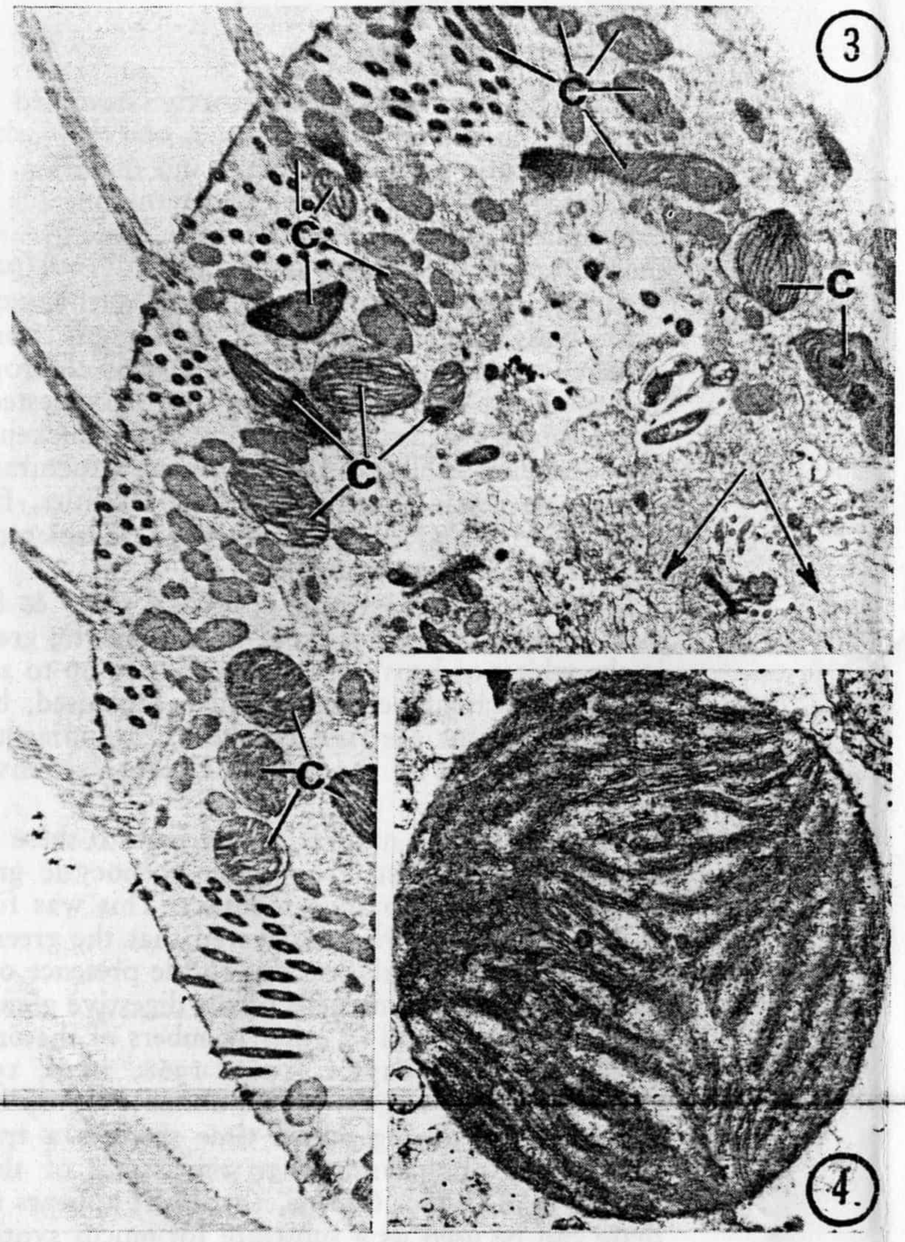


Fig. 3. The periphery of a species of the ciliate genus *Strombidium*, containing numerous chloroplasts (c). Chloroplasts undergoing digestion are located deeper in the cell (arrows), possibly near the cytostome in view of the cilia sectioned in their vicinity. X 3,400.
Fig. 4. A chloroplast from the cytoplasm of a marine ciliate, *Prorodon* sp. X 17,900.

The phenomenon is also known in some comb jellies ctenophore coelenterates; refs. in Nicol, 1960).

If an organism such as a sea slug had acquired chloroplasts on a permanent basis the secondary nature of the phenomenon would be quite evident due to the large phylogenetic gap between these animals and any photosynthetic organism. However, among the eukaryotic protists it would be difficult to recognise such a condition. The presence of chloroplasts, for example, within a basically non-photosynthetic stock (i.e. a dyad, secondarily becoming a triad cell) might well be taken as a primary phenomenon, the

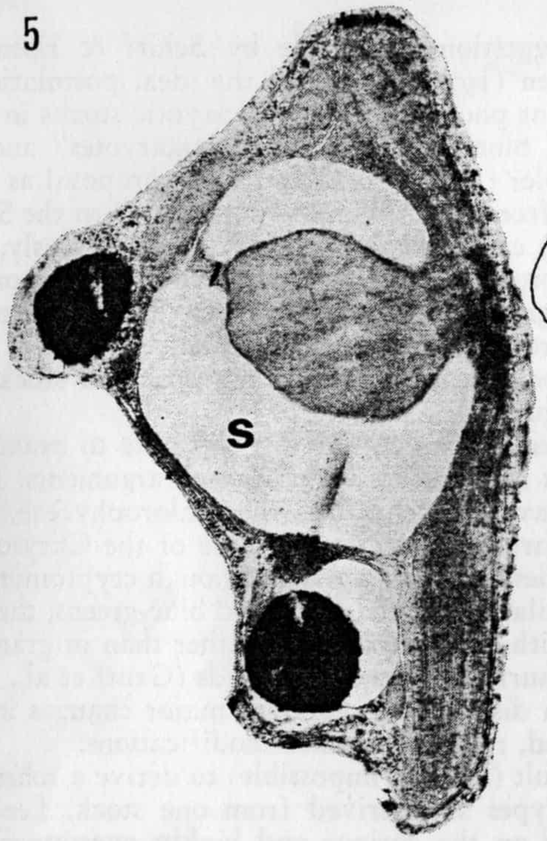
chloroplasts being assumed to have originated within the stock itself. For this reason it is worth reviewing the little data available on temporary foreign organellar maintenance in some marine ciliates and foraminifera, both groups being considered to be basically non-photosynthetic.

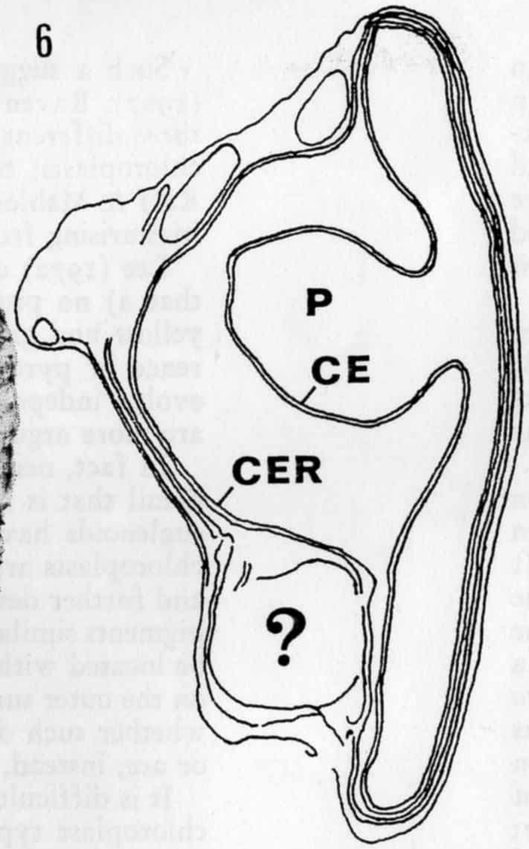
Blackbourn et al. 1973) have discovered that the small, greenish-brown bodies sometimes occurring in some marine planktonic ciliates of the genera *Prorodon* and *Strombidium* are chloroplasts. Structurally they are in very good condition (figs. 3, 4), resembling chloroplasts in their normal state. It has not been possible to identify the algal group(-s) from which these chloroplasts have come as they exhibit one of the most common ultrastructural organisations (the type III of Bisalputra, 1973). It is known to occur in members of the Dinophyceae, Bacillariophyceae, Chrysophyceae or Haptophyceae (the Phaeophyceae also have this type but can be ruled out as a food source, theoretically lacking "unicellular" members). Each foreign chloroplast is surrounded by a double membrane. They do not appear to be located within digestive vacuoles. In one instance a cell of *Strombidium* was observed to be digesting chloroplasts in the vicinity of its cytostome, presumably recently acquired while containing numerous healthy-looking chloroplasts in its periphery Staining with iodine records the presence of many starch bodies within the ciliate hosts.

Some ecological and biochemical data is available on a "red-tide bloom" (population explosion causing discolouration of the sea) caused by one of the *Prorodon* species (Holm-Hansen et al., 1970). It was found that its carbohydrate content (8%) was intermediate between that of marine protozoa and that of photosynthetic plankton, the DNA content (1.2%) being similar to the latter The organisms had 0.23% of chlorophyll *a*, this value representing that unaffected by acid digestion. Chlorophyll *c* could not be found, an unusual discovery in view of its presence in all the potential donor algal groups. A red pigment was found which, however, differed from red phycobilin pigments in not being water soluble. Blackbourn et al. (1973) have concluded that the chloroplasts are derived from photosynthetic food organisms in much the same way as those of the sacoglossan molluscs. However, the residence time of the chloroplasts within the ciliate is unknown.

E. Lanners & K. Grell (pers. comm.) have found chloroplasts from food diatoms in the marine benthic foraminiferan, *Metarotaliella parva*, obtained from the Mediterranean Sea, and chloroplasts have also been seen in other species from the Eilat in the Gulf of Aqaba (Z. Reiss, pers. comm.). There are indications that these chloroplast populations are of greater longevity, but details are not available. As these organisms have been cultured they hold a greater hope for experimentation in the near future than the marine ciliates, which have not been cultured as yet.

A curiously intermediate condition between conventional algal endosymbiosis and chloroplast maintenance seems to be exhibited by the planktonic marine ciliate, *Mesodinium rubrum* c.f. review by Taylor et al., 1971). So-named because of the presence of maroon-brown pigmented bodies, this cosmopolitan species, often a cause of "red tides," has long been assumed to harbour endosymbiotic algae. On the basis of unusual pigment characteristics Parsons and Blackbourn 1968) concluded that the alga belonged to the Cryptophyceae. From field observations on natural occurrences of large numbers, it has been shown that *M rubrum* is a functional autotroph, fixing $^{14}CO_2$ and evolving O_2 (Ryther, 1967; Barber et al., 1969). The





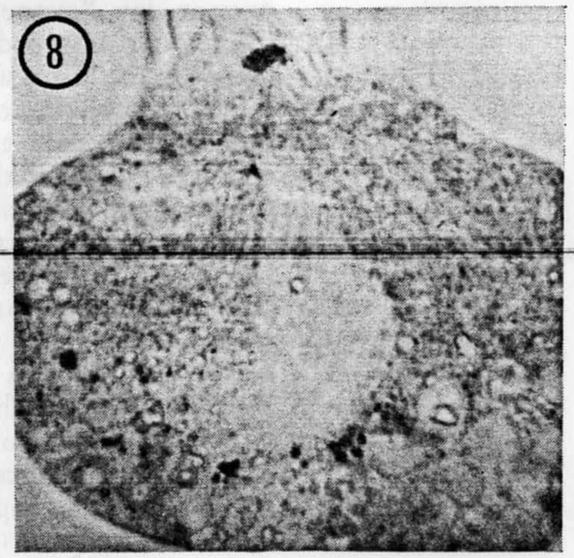


Fig. 5. A chloroplast/mitochondrial complex apparently derived from a cryptomonad within the marine ciliate *Mesodinium rubrum*. S = starch sheath. X 16,400.
Fig. 6. A line interpretation of the principal membrane layers in fig. 5. (CE = chloroplast envelope; CER = chloroplast endoplasmic reticulum; ? indicates the expected position of a cryptomonad nucleus.)
Fig. 7. *Strombidium* c.f. *oculatum* Gruber, a marine ciliate possessing an "eye-spot" (arrow) apparently aggregated from photosynthetic flagellate food. X 440.
Fig. 8. A squash of the cell in fig. 7. Note other carotenoid granules deeper in the cell, far from the eyespot, presumably derived from food. X 440.

organism is extremely difficult to handle, bursting easily when roughly treated or when subjected to routine ecological preservatives other than Lugol's Iodine Solution. It could, however, be prepared for electron microscopy by Taylor et al., 1969). It was found that the pigmented bodies correspond to unusual complexes, termed "incomplete symbionts" (figs. 5, 6). Each consists of a single chloroplast, a stalked pyrenoid, always surrounded by starch, several mitochondria, osmiophilic droplets, ribosomes and some membrane systems, all within an outer double membrane. The ultrastructural details are compatible with a cryptomonad identity However they differ from cryptomonad cells in lacking flagella, the entire peripheral region, all ejectisomes ("trichocysts"), the gullet, and Golgi apparatus. Taylor et al. 1969, 1971 were unable to observe any bodies which might be referrable to a nucleus, despite its predictable location in cryptomonads (the nuclear membrane being confluent with chloroplast endoplasmic reticulum, as indicated earlier) and the examination of a great many of such complexes. They did not, however, use serial sections and therefore could not conclude that the nucleus is truly vestigial or absent. There is a pressing need to determine the time-scale for these complexes. Although unable to establish a permanent culture, Taylor et al. maintained pigmented cells for more than a month in light-equipped incubators.

Some earlier authors, including Bakker 1967), assumed that *M rubrum* was merely a pigmented stage of the colourless *M pulex*. However there are many indications that this is not the case. For example, *M rubrum* shows a high degree of apparent adaptation to its unusual symbiotic state (based on comparisons with other related ciliates and *M pulex* in particular). Its cytostome is degenerate, lacking an oral cone and tentacles. The cells swim backwards, with the cytostome directed posteriorly instead of anteriorly The peripheral structure of the ciliate is greatly reduced so that alveoli, at one time thought to be universal in ciliates, are absent. This thinning (plus strong vacuolation) is probably the reason for its structural delicacy However this is probably a "window effect," permitting increased light penetration. The chloroplasts are always peripherally placed in the ciliate. All the complexes appear to be in a similar state within the cells, and all invariably produce starch about the pyrenoid, often in large quantities. In an unpublished experiment *M pulex* was cultured for several months in the laboratory, being fed several small species of cryptomonad obtained from the same vicinity as *M rubrum*, hoping to bring about a similar condition, but it was not possible to do so, the cryptomonads being rapidly digested.

It is too early to be able to define the complexes from *M rubrum* with certainty However this author suspects that they represent an extreme case of degeneration by an endosymbiotic algal protist. It would be very interesting if it could be shown that the nucleus of the cryptomonad is indeed reduced to a functionless vestige and that the ciliate is now co-ordinating the functions of the complexes. However, the possibility that they are being continually replaced, on a time-scale of weeks or months, cannot be discounted.

Burkholder et al. (1967) have described a ^{14}C-fixing "bloom" of ciliates off Puerto Rico, caused by a different species than those described here. The nature of its pigmented bodies cannot be determined. Norris (1967) has also observed pigmented bodies which he suspected to be chloroplasts within a marine ciliate.

Chloroplasts are probably the easiest organelles to recognise in a foreign situation. However, what about the maintenance of other types of foreign organelles? The ability of mouse fibroblasts to temporarily maintain chicken mitochondria has already been mentioned. Foreign mitochondria would be extremely difficult to recognise visually in view of their relative morphological conservatism, although Taylor et al. (1969, 1971) could distinguish the foreign mitochondria from those of the ciliate in *M. rubrum* by the structure of their cristae.

Some ciliates are known to assemble functional eye-spot conglomerates from ingested algal cells (e.g. *Strombidium oculatum:* Fauré-Fremiet, 1948; figs. 7, 8 here). However the translocation of carotenoid-containing droplets to the anterior of the cells, although interesting enough, cannot rival the sophistication of true organellar maintenance.

The nucleus remains to be examined in this regard. Nuclear dimorphism is well known in some protist groups and has been extensively studied in foraminifera and ciliates (c. f. Grell, 1964; Raikov, 1969 for reviews). It has only recently been discovered in two species of dinoflagellates, and the latter cases appear to differ fundamentally from the former. One of the principal distinguishing features of dinoflagellates is the possession of a nucleus containing unusual, habitually condensed chromosomes (*Noctiluca* and some parasitic species have chromosomes which undergo expanded as well as condensed phases) with very little histone, chromosome separation involving contact with the nuclear membrane in a manner reminiscent of the separation of the bacterial genophore (Leadbeater & Dodge, 1967; Kubai & Ris, 1969). Dodge (1971) and Tomas et al. (1973) have found second, morphologically distinct nuclei to be present in strains of *Glenodinium foliaceum* and *Peridinium balticum* respectively. In the former case the second nucleus is reminiscent of that of a parasitic dinoflagellate (J. Cachon, pers. comm.). In both cases the supernumery nucleus has expanded interphase chromosomes. Tomas & Cox (1973) found, on further examination, that in *P. balticum* the chloroplasts are structurally associated with the non-dinokaryotic nucleus although dispersed throughout the cell, being enveloped by a common membrane. It is not known if the double nuclei of dinoflagellates contain the same information or not. The identity of each nucleus is maintained through the cell cycles. Closely related species apparently lack double nuclei. These features suggest that the non-dinokaryotic nuclei are of foreign origin, being associated with extreme endosymbiotic degeneration perhaps analogous to that found in *M. rubrum.*

The question raised at the beginning of this section remains open. Temporary maintenance in some contemporary organisms seems to be well established, but there do not seem to be any clear examples of permanent maintenance of foreign organelles. In any case, such examples would be very difficult to recognise.

Multiple symbioses for the same type of organelle

An almost inevitable speculation to arise from combining the S.E.T. with observations on heterogeneity within organelles, in particular the diversity of structure and accessory pigments found in algal chloroplasts, is the suggestion that some or most of this heterogeneity may be due to the incorporation of similar but evolutionarily divergent stocks to form similar organelles.

Such a suggestion was made by Schiff & Epstein (1965) and Sagan (1967). Raven (1970) expanded the idea, postulating the involvement of three different photosynthetic prokaryotic stocks in the formation of algal chloroplasts: blue-greens, "yellow prokaryotes" and "green prokaryotes." Raff & Mahler (1972) described such a proposal as one of the improbabilities arising from the "dogmatic adherence" to the S.E.T.

Lee (1972) criticised the proposal more seriously, his main points being that a) no prokaryotes are known which correspond to the hypothetical yellow and green prokaryotes; b) that Raven did not consider the occurrence of pyrenoids in all three types; and c) that chlorophyll *c* had to evolve independently in two of the proposed stocks. His other criticisms are more arguable.

In fact, neither Raven nor Lee was able to examine chloroplasts in the detail that is required to evaluate such arguments. For example, although euglenoids have pigments like the Chlorophyceae, ultrastructurally their chloroplasts are more similar to those of the Chrysophyceae (Gibbs, 1970, and further details below). Also, although cryptomonads possess phycobilin pigments similar to the red algae and blue-greens, the phycobilins appear to be located within the thylakoids, rather than in granular "phycobilisomes" on the outer surfaces of the thylakoids (Gantt et al., 1971). It is not known whether such distinctions represent major changes in metabolic machinery or are, instead, relatively minor modifications.

It is difficult (but not impossible) to derive a scheme whereby all known chloroplast types are derived from one stock. Lee has produced such a scheme based on the curious and highly questionable hypothesis that, as present day "cyanelles" (blue-green endosymbionts and structures which seem to be intermediate between blue-greens and chloroplasts: see further details in the discussion section) occur most commonly in *Glaucocystis* and its relatives, thought to have been derived from apochlorotic cryptomonads, this indicates that it was in cryptomonads that the first chloroplasts evolved. This assumes that these present day symbioses have maintained a similar state for a thousand million years or more!

Most recent information continues to re-inforce the considerable similarity between blue-greens and red algal chloroplasts. Not only is the general pigment spectrum and thylakoid arrangement similar, and the phycobilin pigments located in phycobilisomes on the outer surfaces of the thylakoids, but it has also been shown that there is immunological similarity between the phycobilins of the two groups (eg. Glazer et al., 1971) but not between those of the cryptomonads and the other two (Glazer & Cohen-Bazire, 1971). This evidence strongly suggests a direct evolutionary link between the red algal chloroplast and blue-greens, although not necessarily between entire red algal cells and blue-greens as advocated by many earlier authors such as Dougherty & Allen (1960), Klein & Cronquist (1967) and Allsopp (1969).

These types of observations underline, more strikingly than ever, the co-occurrence of highly primitive features (determined by their resemblance to prokaryotes) in one type of organelle with relatively advanced features in another within the algae. Thus the dinoflagellates seem to exhibit a primitive nuclear arrangement, but have chloroplasts which are not particularly primitive. Red algae have chloroplasts that appear to be primitive, possibly accompanied by a primitive lack of flagellation (Dougherty & Allen, 1960), and a mitotic mechanism somewhat less primitive to the

dinoflagellates, the spindle penetrating the nucleus from extra-nuclear loci (McDonald, 1972). Cryptomonads do not appear to be particularly primitive in any regard other than an apparent lack of sexuality and most closely resemble the Chrysophyceae despite their pigment spectrum and paired thylakoids (Oakley & Dodge, 1973).

It is evidently too soon to make a realistic assessment of this problem. The lack of congruence of phylogenies based solely on chloroplasts with those based on other characters raises doubts as to the use of chloroplast features as primary features by which photosynthetic groups may be recognised and systematically arranged. In particular, the euglenoids illustrate the weakness of the use of photosynthetic pigments as pre-eminent phyletic characters. Their possession of chlorophylls a and b, plus other accessory pigments in common with the green algae has long been considered indicative of a close relationship. However, not only do their chloroplasts differ ultrastructurally (as indicated above), but euglenoids differ from green algae in their nuclear structure and mitotic mechanism, cell wall composition, location and structure, flagella, paraflagellar swelling, canal, reservoir, eyespot location, reserve product and its location, presence of muciferous bodies, trichocysts (in some only), symmetry, euglenoid movement and apparent absence of sexuality (c.f. review by Leedale, 1967).

The conservativeness of chloroplast features *within* the algal groups (Gibbs, 1970; Bisalputra, 1973) indicates that a considerable amount of co-evolution has occurred, and that the acquisition of chloroplasts by these groups occurred a long time ago.

The question of the possibility of new types of chloroplasts being produced by symbioses at the present time (eg. cyanelles) will be returned to in the concluding section.

Discussion

This contribution has concerned itself primarily with aspects of the S.E.T. because it is the latter view of the origin of eukaryotic structure which is rapidly becoming the yard-stick against which other theories are tested. There are several probable reasons for this.

Firstly, comparative information which has been obtained in the past decade concerning mitochondria, chloroplasts and prokaryotes has indicated a greater structural and functional similarity between the organelles and entire prokaryotic cells than between them and the cell which surrounds them, particularly with regard to the mechanisms of protein synthesis.[2]

The evidence bearing on this has been recently reviewed by many including M. Nass (1969*b*, 1971), S. Nass (1969), Wagner (1969), Carr & Graig (1970), Cohen (1970, 1973), Echlin (1970), Stanier (1970), Raven (1970), Margulis (1970), Taylor (1970), Wilkie (1970), Luft (1971), Schnepf & Brown (1971), Whitton et al. (1971), Hall (1973), Uzzell & Spolsky (1973) and Green (1973).

Meyer (1973) indicated that mitochondrial DNA has greater similarities to bacterial plasmid DNA than to the genophore DNA, considering its smaller size, physical properties, mode of replication and sensitivity to acridine dyes and ethidium bromide. Assuming that no major changes have occurred since the formation of the first mitochondria (the S.E.T. requires the transfer of genetic control for many functions to the host nucleus, with a consequent reduction of the organellar genome) this tends to support the alternative view that mitochondria may have formed from the association and isolation of plasmid DNA with invaginations of the plasma membrane although it could be explained by the S.E.T. as well. Haldar et al. (1966) had already proposed a mesosomal type of origin for mitochondria, Gibor (1967) discussing the possibility further. Another explanation of the lesser amount of genetic autonomy found in mitochondria than in chloroplasts is that they have been in symbiotic association longer and have consequently become more integrated with their hosts in all respects.

Allsopp (1969) and Raff & Mahler (1972, 1973) are the only recent authors to oppose the S.E.T. strongly, and the latter are the only to suggest a detailed alternative mechanism for the origin of mitochondria, essentially similar to the views of Meyer and Haldar et al. in that it involves the enclosure of a plasmid within a metabolically specialised sac. No particular reason was given for the advantage this may impart to a cell, other than additional permeability control.

Raff & Mahler have made much of evidence suggesting the fundamental aerobic nature of the encompassing cell (presence of superoxide dismutase as an oxidation protectant, plus the exclusively aerobic synthesis of unsaturated fatty acids and steroids), claiming that these are indications that the protoeukaryote was already an aerobic respirer and thus would receive no selective advantage from the incorporation of another with an aerobic respiration system. However, they do not discuss the necessity for the protoeukaryote to survive in micro-aerobic environments in order for it to have been able to encounter aerobic bacteria with which to form a symbiosis. Furthermore, Hall (1973) has presented arguments for the advantageousness of the existence of mitochondria, irrespective of whether the surrounding cell was anaerobic or not and de Duve (1973) has proposed that the host, although aerobic, may have had a primitive peroxisomal type of respiration.

Caution is required in distinguishing the advantages presented to hosts by endosymbionts. For example, although the occurrence of photosynthetic endosymbionts within photosynthetic hosts is rare, it is not unknown. In diatoms two species of blue-green bacteria occur endosymbiotically, one in *Rhopalodia gibba* (Drum et al., 1966) and the other (*Richelia intracellularis*) in various marine planktonic centric diatoms, especially members of *Rhizosolenia* (Hustedt, 1930 and personal observations). At first sight there seems to be no advantage in these associations. However, as the *R. gibba* blue-green consortium is capable of thriving in media in which there is no nitrogen source other than N_2, it seems probable that the advantage has nothing to do with photosynthesis, but is instead concerned with nutrient availability. The relationship of *Richelia* with its hosts is not known as yet.

The other principal criticism of the S.E.T. by Raff & Mahler is that it requires a "wholesale transfer of genes from the endosymbiont genome to an unrelated nuclear genome." They point out that such a mechanism is "difficult to conceive," although perhaps one of the most exciting areas of modern genetics is the transfer of bacterial genes to the nucleus of a higher

2. An example is provided by the study of Pigott & Carr (1972). Using nucleic acid hybridisation in *Euglena gracilis*, they found values of 8.7 to 47% between chloroplast DNA and blue-green rRNA, with lower values for rRNA from bacteria (1.1 to 4.5%) and between chloroplast DNA and *Euglena* cytoplasmic rRNA (1%).

plant: *Arabidopsis* (Ledoux & Huart, 1971). In the *Mesodinium rubrum* photosynthetic consortium described earlier there exists a possibility that, if the foreign chloroplasts and mitochondria are permanently maintained (not yet established) genes for their regulation may have passed from the cryptomonad symbiont to the ciliate host.

The mitochondrial origin model by Raff & Mahler has several awkward features. For example, they begin with a protoeukaryote possessing a double outer membrane, the inner membrane bearing the respiratory assemblies, not indicating what type of prokaryote this might be (Gram negative bacterium?). The mitochondrial precursors are at first unable to maintain themselves, being continually reformed from the general respiratory membrane, until they fortuitously capture a plasmid bearing precisely the right genes for the appropriate protein synthesis needed to sustain them. After this a second membrane surrounds the organelle by no specific mechanism and for no obvious selective advantage. As indicated later in this section it is also unrealistic to discuss the origin of mitochondria independently from that of chloroplasts.

Cells with a general organisation intermediate between entire prokaryotic and eukaryotic cells have not been found. This does not prove that such cells do not exist or may not have existed a long time ago. However, such a discovery would be higly damaging to the S.E.T. for, as Margulis (1970) has stressed, such a state is inconsistent with the S.E.T. Instead it has become increasingly clear that among some of the states termed "syncyanoses" (Pascher, 1914, 1929) formerly interpreted as associations of endosymbiotic blue-green bacteria (cyanelles) with eukaryotic hosts there are several in which the pigmented bodies are so intermediate in appearance that it is entirely an arbitrary decision at present as to whether they are blue-green cells or chloroplasts whereas others are clearly blue-greens. This is particularly the case for the bodies within the cryptomonad *Cyanophora paradoxa* and the coccoid alga of uncertain affinities (possibly Chlorophycean), *Glaucocystis nostochinearum* (see reviews by Taylor, 1970; Margulis, 1970, and Schnepf & Brown, 1971). Stanier (1970) has indicated how the criteria for the determination of cyanelles from chloroplasts, proposed by Pringsheim (1958) and Geitler (1959), are inadequate for the task of dealing with the latter two species.

Hovasse (1965) and Hovasse et al. (1967) have drawn attention to the resemblance between the coiled ejectile bodies of the trichocysts ("taeniobolocysts") of cryptomonads and the R bodies of "kappa particles", bacteria living within *Paramecium aurelia* (see review by Beale et al., 1969). It could be suggested that the former have had a xenogenous origin, being products of bacteria which have subsequently degenerated to an unrecognisable state and become fully integrated with the host. The observation of Schuster (1968) that some cryptomonad trichocysts can be eliminated by the use of antibiotics, and could not be reformed in bacteria-free culture, seems to support this possibility. However, unpublished observations by this author indicate that the cryptomonad *Chroomonas salina* does not lose its trichocysts in axenic culture. Furthermore Mignot et al. (1970) and Preer et al. (1972) have found that the ejectile ribbons extend in several different ways in the ciliate endosymbionts whereas in cryptomonads they always form a unique bi-partite, non-tubular rod. The cryptomonad trichocysts occur in sacs isolated from the cytoplasm by a single membrane, rather than the double membrane usually associated with endosymbionts, and

seem to originate in the vicinity of the Golgi apparatus (Anderson, 1962). Schuster (1968) found an apparent labelling of some trichocysts and the Golgi region by tritiated thymidine, but his figures illustrating label are not compelling indications of the presence of DNA in these structures, label occurring outside the cell, and not in most trichocysts or the nucleus. This aspect should be examined in more detail.

An intriguing further possibility has been raised by Preer et al. (1972). In view of the invariable presence of phage-like structures with R bodies they have suggested that the ejectile ribbons are, in fact, products of phage activity in the bacteria within the ciliates. Phages have not been seen in cryptomonad trichocysts.

The difficulty which has been experienced in determining whether or not intracellular bodies resembling prokaryotes are endosymbionts or organelles is a further point in favour of the S.E.T. One may well ask just what is it that distinguishes an obligate intracellular endosymbiont from an organelle? For example, mitochondria and chloroplasts possess nearly all the properties listed by Beale et al. (1969) as being indicative of the bacterial nature of the variety of different intracellular "particles" in *Paramecium aurelia*, lacking only the presence of diaminopimelic or muramic acids, and infectivity. The ability to grow persistently in host-free culture conditions might be a good index of the degree of metabolic interdependence existing between the components, but the claims by Giles & Sarafis (1972) of repeated division observed in cultures of chloroplasts in hen's eggs show that even this can only be a matter of degree rather than being an absolute criterion. Much has been made of the fact that mitochondria and chloroplasts are only partially autonomous. In one sense this is a *non-sequitur* for, if they were fully autonomous, they would be facultative endosymbionts.

Metabolic interdependence is a common property of intracellular symbioses. Just how rapidly this phenomenon can arise has been recently shown by Jeon (1972) during micrurgical transplantation studies on amoebae. He found that a strain of *Amoeba discoides* not only became resistant to a type of infective bacterium but, after five years of infected growth, also acquired a nuclear dependence on the bacterium. Only 7 percent of those individuals in which nuclei from infected cells were implanted in bacteria-free cells were able to produce viable clones, in comparison with 91 percent for the reciprocal combination. The nature of the dependence is not known.

It would seem that, from a practical point of view, if an intracellular structure is an obligate component of the cell (neither component being able to get along without the other), then it is an organelle of that cell, regardless of its origin. Advocates of autogenous theories might take exception to this view, however.

A major point in favour of the S.E.T. at present is that it has received detailed exposition and makes many assertions which can be tested by examining contemporary organisms. Alternative ideas have been very inadequately presented by comparison, and fall largely into the category of the *ad hoc* evolutionary theories deplored by Lewontin (1972), although not necessarily incorrect.

One point on which the various theories agree at present (summarised in fig. 9) is that the considerable uniformity, together with structural complexity of the eukaryotes, makes it highly unlikely that they have a poly-

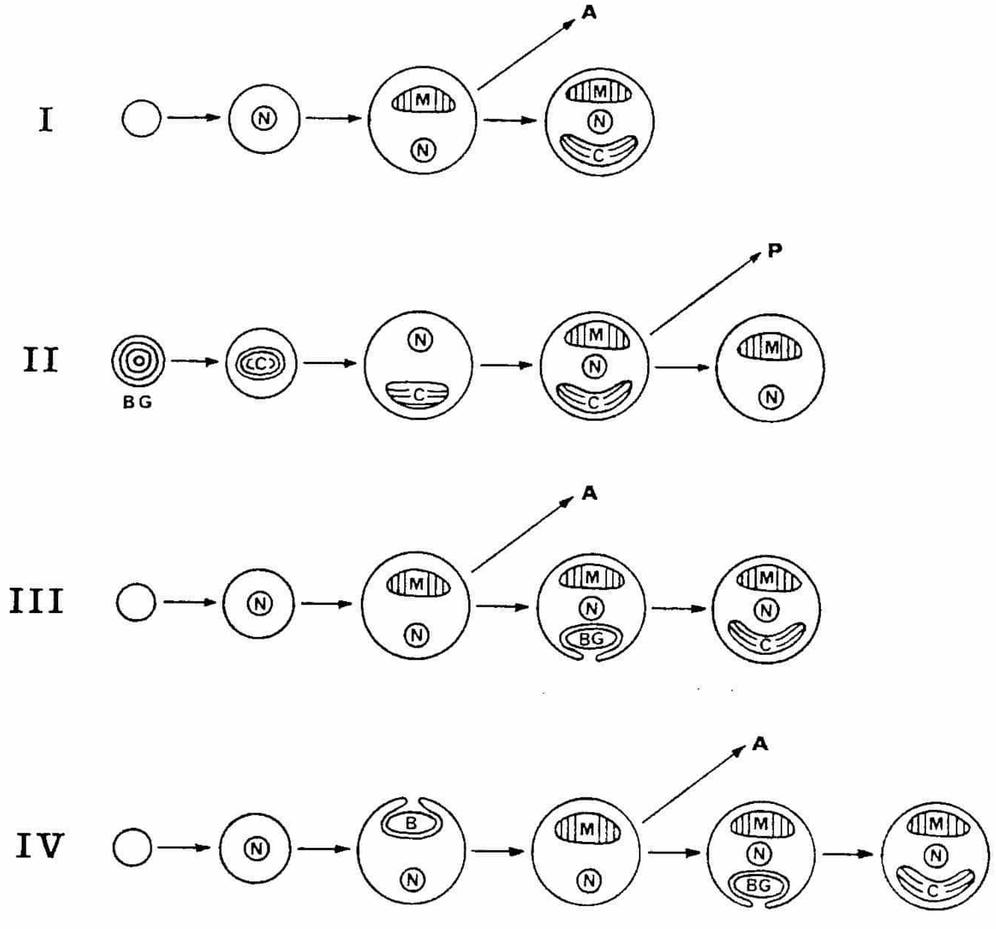


Fig. 9. A diagrammatic summary of the principal alternatives proposed for the origin of nuclei, chloroplasts and mitochondria. I. The fully autogenous formation of the three types of organelles from a non-photosynthetic ancestral prokaryote. II. Origin of eukaryotes from a photosynthetic prokaryote (the "Uralga" theory). III. The autogenous origin of the nucleus and mitochondria, with the symbiotic acquisition of the chloroplast. IV. The symbiotic acquisition of both mitochondria and chloroplasts. "A" and "P" indicate lines proceeding to animal-like or plant-like organisms respectively.

phyletic origin. Furthermore, the near ubiquity of flagella among the eukaryotic groups suggests that flagella (and by essential association, organised microtubular growth) developed early in the evolution of the eukaryotes. The development of the nucleus in association with elaboration of the endomembrane system (eg. Robertson, 1964) is also accepted by most authors, including advocates of the S.E.T. Goksøyr (1967) suggested that the increase in nuclear DNA arose by a coenocytic fusion of prokaryotic precursor cells, and Sonea (1972) thought this may have occurred by the accumulation of plasmids although neither of these postulated events seems necessary to account for the development of multiple chromosomes.

A major point of disagreement concerns the nature of the initial eukaryotic stock. In Margulis' view (Sagan, 1967) this was a non-photosynthetic, fermentative cell which developed an amoeboid form of ingestion (phagocytosis) after the incorporation of the protomitochondrion. She views some of the giant amoebae as the most primitive of contemporary

protists although nuclear characteristics suggest that dinoflagellates might also be candidates for this position.

The most commonly expressed opposing view is that the first eukaryote was a photosynthetic organism, the "Uralga" (Klein & Cronquist, 1967; Allsopp, 1969). The latter stems chiefly from the strong similarity between blue-greens and red algae (or, more precisely, red algal chloroplasts) in thylakoid arrangement, pigment array including distinctive phycobilin pigments, occurrence of the latter phycobilins within granular "phycobilisomes" located on the outer surfaces of the thylakoids, and the production of α1-4 polyglucan reserve products. Holders of such a view postulate the direct evolution of eukaryotes from a blue-green-like prokaryotic stock, (option II, fig. 9) and consider the red algae as the most primitive contemporary eukaryotic group.

It is indeed difficult to imagine two such similar photosynthetic structures arising in widely separated stocks. Stanier (1970) favours the view that all photosynthetic processes have arisen from a single ancestral line. This is a strong argument against any fully autogenous theory other than that of the Uralga (option I, fig. 9). It is not reasonable to discuss only the autogenous origin of the mitochondrion, as Raff & Mahler (1972, 1973) or de Duve (1973) have done. Unless one advocates an extraordinary evolutionary parallelism of photosynthetic systems, the origin of the mitochondrion must be considered together with the chloroplast, even though the latter is not of universal occurrence in the eukaryotes. The effect of this constraint is that the autogenous development of the mitochondrion must be considered as occurring within a blue-green-like ancestral cell or a eukaryotic derivative of it.

If eukaryotes arose directly from blue-green-like ancestors it is the chloroplast which is the primordial remnant. This proposal requires the separation of genetically distinct material to form mitochondria and the eukaryotic surrounding entity (termed the "80S" matrix, using the ribosomal sedimentation property as a convenient label). In view of the prokaryote-like features shared by mitochondria and chloroplasts it is more likely that mitochondria were formed from the blue-green material before the remainder. However, their location within the eukaryotic "80S" matrix, intimately related to the endoplasmic reticulum system, seems contrary to this. Alternatively, the blue-green chloroplast became surrounded by the 80S matrix first (option II, fig. 9), with the formation of a nucleus and the mitochondria later in conjunction with the development of membranes. But this requires that there was a separation of an 80S genophore from the 70S one normal to blue-greens followed by reversion of part of the latter to a 70S type with the formation of the mitochondria. All in all, either alternative seems very doubtful. In Allsopp's (1969) variation, the eukaryotic nucleus formed *within* a blue-green-like ancestor, although he did not elucidate any further stages in the eukaryotic development.

Stanier (1970), although he favoured the S.E.T., considered it more probable that the chloroplast was formed (by symbiosis) before the mitochondrion. He gave as his reason evidence that aerobic respiration must have occurred long after photosynthetic organisms had begun to produce appreciable quantities of oxygen. In fact, assuming this to be so, there seems to be no reason why this production of oxygen could not have been entirely due to free-living blue-greens. The universal presence of mitochondria in eukaryotes (other than in a few cases of obvious secondary loss),

exhibiting much less diversity than chloroplasts, and seeming to show a greater integration with, and dependence on, the host cell, are other arguments in favour of the early establishment of the mitochondrion and much later establishment of chloroplasts, also being essential for the increase in size and active endocytotic production required by the early eukaryotes (Margulis, 1970).

Further support for the view that the initial eukaryotic stock was non-photosynthetic (thus also being contrary to the Uralga theory) comes from recent cytocrome *c* sequence data (McLaughlin & Dayhoff, 1973) indicating that non-photosynthetic eukaryotic protists, such as the trypanosomes, are closer to recent prokaryotes than the photosynthetic eukaryotes. However, this is based on data from only one non-photosynthetic protist, *Crithidia oncopelti*.

The remaining principal alternative (option III in fig. 9) is that the mitochondrion has had an autogenous origin, whereas the chloroplast had a symbiotic origin. Such a proposal might satisfy those whose principal criticism of the S.E.T. centres on the relatively small degree of genetic independence exhibited by mitochondria, and the resemblances of their DNA to plasmid rather than bacterial genophore DNA (Meyer, 1973). While being quite possible, proponents of such a view will have to elaborate on the reasons for the strong similarities between mitochondria and chloroplasts (including the similar topological relationship referred to earlier). To this author this is the second most attractive option after the S.E.T.

Although not included in fig. 9 mention must be made of the proposal by S. Nass (1969). He suggested that eukaryotes may have arisen by the fusion of a colonial prokaryote in which the differentiation of individuals for specialised functions and the concentration of genetic material within some members had already occurred. There seem to be no particularly compelling arguments in favour of this view which, according to Stanier (1970) has "the distinct flavour of science fiction". Similarly there does not seem to be any particular reason to invoke the coenocytic fusion of similar prokaryotic individuals in order to obtain the added DNA needed for eukaryotic chromosomes as Goksøyr (1967) proposed in a theory which was otherwise identical to that of Sagan (1967) but not documented to any extent.

As yet little mention has been made of a further almost universal constituent of eukaryotes: microtubule organising centres (MTOC's in the abbreviation of Pickett-Heaps, 1969). These enigmatic bodies, of which centrioles and basal bodies (Margulis' "9 + 2" homologues) are the most highly organised expression, appear to be essential for the production of microtubules, and in turn, for the guidance and control of many uniquely eukaryotic activities, including mitosis and flagellar/cilia development and movement. The crucial importance of the microtubular system to the evolution of the eukaryotes has been stressed and discussed by both Margulis (1970) and Stanier (1970), with, however, differing suggestions as to the origin of the system.

Noting the existence of several contemporary examples of flagellates, particularly in the rumen of cattle in which propulsion is achieved not by the flagella but by the contractions of thousands of ectosymbiotic spirochaetes, Margulis (1970, and as Sagan, 1967) developed the theory that motility was imparted by the formation of a similar type of endosymbiosis. This eventually led to the formation of the typical eukaryotic flagellum

with its centriole-like basal body, and internal microtubular elaboration (and centrioles free of the basal body) developed from this, leading to spindle formation and the reliable method of chromosomal separation into daughter nuclei essential for further development of the eukaryotes.

Pickett-Heaps (1969) has produced an alternative proposal which challenges the conventional view of the primacy of the centriole in the organisation of microtubular systems. Instead he has suggested that the poorly defined loci around which microtubules undergo self-assembly in many organisms, in particular plants, are primordial MTOCs. In a more elaborate form they can be seen with the electron microscope as electron dense globules, plaques or rings. In his view centrioles or basal bodies are the product of MTOCs, rather than being the primary progenitors. He has pointed out that even in centriole-associated spindles there is a dense, granular zone interposed between the centriole and the microtubules, this zone possibly being the MTOC. Only in the case of flagella, where the nine outer doublet microtubules are extensions of members of the peripheral triplets in the basal body, is there direct continuity between these barrel-shaped bodies and microtubules. The assembly of new centrioles or basal bodies from the general vicinity of existing ones, rather than by fission of parent organelles (Dingle & Fulton, 1966; Kalnins & Porter, 1969), as well as *de novo* formation of basal bodies in cases of zoosporogenesis or amoebo-flagellate transformation (eg. *Chara*, *Naegleria*), indicate a fundamental distinction between them and other organelles thought to have originated from organismal endosymbioses. The absence of a delimiting membrane referred to earlier in the topological section (and also by Schnepf & Brown, 1971) is another distinction. The presence of DNA within basal bodies or centrioles has been a vexed question (eg. Randall & Disbrey, 1965; Smith-Sonneborn & Plaut, 1967; Dippell, 1968; Sonneborn, 1970), DNA from closely adjacent mitochondria leading to discriminatory difficulties. Recent studies on organisms producing great numbers of basal bodies in a short period of time, such as in the formation of oral membranellar bands in the ciliate *Stentor*, indicate that no measurable DNA synthesis accompanies the process (Younger et al., 1972).

It is concluded here that the S.E.T. seems to offer the most plausible explanation for the origin of mitochondria and chloroplasts. On the other hand, Pickett-Heaps' (1969) interpretation of MTOCs, which is an attractive one to this author, renders the xenogenous origin of centrioles or other "9 + 2 homologues" doubtful.

It must be admitted that a great deal of the attractiveness of the S.E.T. stems from the great detail in which the theory has been proposed and examined, and there is a serious need for opponents of the theory to produce detailed alternatives, bearing in mind the apparent restrictions drawn attention to here.

One by-product of the S.E.T. is the focus which it has thrown on neglected but crucial aspects of the evolution of eukaryotes. Stanier (1970) has drawn attention to the key roles played by dynamic membrane elaboration and directional structural control through microtubules, aspects which need further critical examination. The evolution of "80s" eukaryotic ribosomes from "70s" prokaryotic ribosomes, or both from some common ancestor, are questions which are obviously also of major importance to the understanding of the unique properties of the eukaryotes.

Another effect of the S.E.T. is that it makes phylogenetic speculation

based on single criteria ("phylogenetic markers") more questionable than ever. It becomes essential to try to trace the history of the component monads independently of each other if possible, to distinguish between that which may have occurred before and after the symbiotic events which brought them together. The "embracer", or host monad becomes the only conservative eukaryotic component. Presumably its history can be investigated by examining features such as the subunits of the ribosomes. Kimura & Ohta (1973) have estimated the time of evolutionary divergence between the 5s ribosomal RNA subunits of pro- and eukaryotes (using sequencing data), combined with information available for cytochrome *c*, concluding that it occurred approximately 1.8 x 10^9 years ago. The latter cytochrome has attracted the interest of several investigators. It is known that the structural gene for it is located within the nucleus, and not within the mitochondrion and so McLaughlin & Dayhoff (1973) have claimed that it is a reliable label for the host monad. However, this might have been one of the genes involved in the transfer from endosymbiont to host genome so doubted by Raff & Mahler (1972).

The mitotic apparatus would seem to be a reliable host feature, as would be flagellar structure (eg. Manton, 1965), but most of the apparent diversity in these cell features may have occurred after the establishment of the primary endosymbioses.

A further effect of the S.E.T. is that it widens the phylogenetic chasm between prokaryotes and eukaryotes even further (as emphasised in the section on levels of organisation). This is an argument in favour of the recognition of a very different two-kingdom system from that usually recognised (see the accompanying article by Leedale): prokaryotes and eukaryotes, rather than plants and animals.

The possibility of the acquisition of organelles by eukaryotes later than the Precambrian does not seem to have been considered before, and yet the existence of "cyanelles" showing an apparently intermediate condition between entire blue-greens and chloroplasts seems to suggest that the conversion of prokaryotes to eukaryotic organelles is a continuing process. It does not seem to be reasonable to suggest that it has occurred only during the Precambrian and the present, or that these present-day conditions have persisted in arrested transition since the Precambrian. In addition to the continuance of such primary phenomena, the peculiar endosymbioses found recently in some marine ciliates suggest that even organellar exchange between unrelated eukaryotic stocks may be possible.

Whatever the outcome of future investigations there is no doubt that the S.E.T. has already stimulated a lively and overdue examination of a major aspect of the evolution of life on earth. It is hoped that this contribution has made some of its implications clearer, and that the unusual, new symbiotic possibilities which have been revealed will not be viewed as the contents of Pandora's Box let loose upon Promethean science.

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References

ALLSOPP, A. 1969 – Phylogenetic relationships of the procaryotes and the origin of the eucaryotic cell. New Phycologist 68: 591-612.

ALTMAN, R. 1890 – Die Elementarorganismen und ihre Beziehung zu den Zellen. Leipzig: Veit & Comp.

ANDERSON, E. 1962 – A cytological study of *Chilomonas paramecium* with particular reference to the so-called trichocysts. J. Protozool. 9(4): 380-395.

BAKER, J. R. 1948 – The status of the protozoa. Nature 161: 548-551.

BAKKER, C. 1967 – Massale ontwikkeling van ciliaten met symbiotische algen in het Veerse Meer. Levende Natuur. 70: 166-173.

BARBER, R. T., WHITE, A. W. and SIEGELMAN, H. W. 1969 – Evidence for a cryptomonad symbiont in the ciliate, *Cyclotrichium meunieri*. J. Phycol. 5: 86-88.

BEALE, G. H., JURAND, A. and PREER, J. R. 1969 – The classes of endosymbiont of *Paramecium aurelia*. J. Cell Sci. 5: 65-91.

BERNHARD, W. and ROUILLER, C. 1956 – Close topographical relationship between mitochondria and ergastoplasm of liver cells in a definite phase of cellular activity. J. Biophys. Biochem. Cytol. 2 Suppl.: 73-77.

BISALPUTRA, T. 1973 – Plastids. *In:* Stewart, W. D. P. [ed.] Physiology and Biochemistry of Algae. Blackwell. Chapter 4. In press.

BISALPUTRA, T. and BAILEY, A. 1973 – The fine structure of the chloroplast envelope of a red alga, *Bangia fusco-purpurea*. Protoplasma 76: 443-454.

BLACKBOURN, D. J., TAYLOR, F. J. R. and BLACKBOURN, J. 1973 – Foreign organelle retention by ciliates. J. Protozool. 20(2): 286-288.

BOUCK, G. B. 1965 – Fine structure and organelle associations in brown algae. J. Cell Biol. 26: 523-537.

BURKHOLDER, P. R., BURKHOLDER, L. M. and ALMODOVAR, L. R. 1967 – Carbon assimilation of marine flagellate blooms in neritic waters of southern Puerto Rico. Bull. Mar. Sci. Gulf Caribbean 17: 1-15.

CARR, N. G. and CRAIG, I. W. 1970 – The relationship between bacteria, blue-green algae and chloroplasts. *In:* Harbonne, J. B. [ed.] Phytochemical Phylogeny; proceedings of the Phytochemical Society Symposium, Bristol, April, 1969. Academic Press, New York.

COHEN, S. S. 1970 – Were mitochondria and chloroplasts microorganisms? Amer. Sci. 58: 281-289.

COHEN, S. S. 1973 – Mitochondria and chloroplasts revisited. Amer. Sci. 61: 437-445.

COHEN-BAZIRE, G. 1971 – The photosynthetic apparatus of procaryotic organisms. *In:* Harris, P. J. [ed.] Biological Ultrastructure: The Origin of Cell Organelles. Oregon State University Press, Corvallis: 65-90.

CORLISS, J. O. 1957 – Concerning the "cellularity" or acellularity of the protozoa. Science 125: 988-989.

CROTTY, W. J. and LEDBETTER, M. C. 1973 – Membrane continuities involving chloroplasts and other organelles in plant cells. Science 182: 839-841.

DINGLE, A. D. and FULTON, C. 1966 – Development of the flagellar apparatus of *Naegleria*. J. Cell Biol. 31: 43-54.

DIPPELL, R. 1968 – The development of basal bodies in *Paramecium*. Proc. natn. Acad. Sci., U.S.A. 61: 461.

DOBELL, C. C. 1911 – The principles of protistology. Archiv für Protistenkunde 23: 269-310.

DODGE, J. D. 1971 – A dinoflagellate with both a mesocaryotic and a eucaryotic nucleus. I. Fine structure of the nuclei. Protoplasma 73: 145-157.

DOUGHERTY, E. C. and ALLEN, M. B. 1960 – Is pigmentation a clue to protistan phylo-

geny? *In:* Allen, M. B. [ed.] Comparative Biochemistry of Photoreactive Systems Academic Press, New York and London: 129-144.
Drum, R. W., Pankratz, H. S. and Stoermer, E. F. 1966 — Electron microscopy of diatom cells. *In:* Helmcke, J. H. and Krieger, W., Diatomeenschalen im Elektronenmikroskopischen Bild, 6 (plates 588-593). J. Cramer, Lehre.
de Duve, C. 1973 – Origin of mitochondria. Science 182: 85.
Echlin, P. 1966 – The cyanophytic origin of higher plant chloroplasts. Br. phycol. Bull. 3: 150-151.
Echlin, P. 1970 – The photosynthesis apparatus in prokaryotes and eukaryotes. *In:* Charles, H. P. and Knight, B. D. [eds.] Organization and Control in Prokaryotic and Eukaryotic Cells, Vol. 20. 20th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge, England: 221-248.
Famintzin, A. 1907 – Die Symbiose als Mittel der Synthese von Organismen. Biologisches Centralblatt 27(12): 353-364.
Fauré-Fremiet, E. 1948 – Le rythme de marée du *Strombidium oculatum* Gruber. Bull. France Belg. 82: 3-23.
Gantt, E., Edwards, M. R. and Provasoli, L. 1971 – Chloroplast structure of the Cryptophyceae. Evidence for phycobiliproteins within intrathylakoidal spaces. J. Cell Biol. 48: 280-290.
Geitler, L. 1959 – Syncyanosen. *In:* Ruhlands Handbuch der Pflanzen-physiologie. Vol. 11. Berlin, Gottingen, Heidelberg: 530-545.
Gibbs, S. P., 1970 – The comparative ultrastructure of the algal chloroplast. Ann. N.Y. Acad. Sci. 175: 454-473.
Gibor, A. 1967 – Inheritance of cytoplasmic organelles. *In:* Warren, K. B. Formation and fate of cell organelles. Academic Press, New York-London: 305-316.
Giles, K. L. and Sarafis, V. 1971 – On the survival and reproduction of chloroplasts outside the cell. Cytobios 4(13): 61-74.
Giles, K. L. and Sarafis, V. 1971 – On the survival and reproduction of chloroplasts out- New Biol. 236: 56-58.
Glazer, A. N. and Cohen-Bazire, G. 1971 – Subunit structure of the phycobiliproteins of blue-green algae. Proc. nat. Acad. Sci. (Wash.) 68: 1398-1401.
Glazer, A. N., Cohen-Bazire, G. and Stanier, R. Y. 1971 – Comparative immunology of algal biliproteins. Proc. nat. Acad. Sci. 68(12): 3005-3008.
Goksøyr, J. 1967 – Evolution of eucaryotic cells. Nature 214: 1161.
Green, B. R. 1973 – Nucleic acids and their metabolism. *In:* Stewart, W. D. P. [ed.] Physiology and Biochemistry of Algae. Blackwell. In press.
Greene, R. W. 1970a – Symbiosis in sacoglossan opisthobranchs: symbiosis with algal chloroplasts. Malacologia 10(2): 357-368.
Greene, R. W. 1970b – Symbiosis in sacoglossan opisthobranchs: translocation of photosynthetic products from chloroplast to host tissue. Malacologia 10(2): 369-380.
Greene, R. W. and Muscatine, L. 1972 – Symbiosis in sacoglossan opisthobranchs: photosynthetic products of animal-chloroplast associations. Marine Biology 14(3): 253-259.
Grell, K. G. 1964 – The protozoan nucleus. *In:* Brachet, J. and Mirsky, A. [eds.] The Cell (Biochemistry, Physiology, Morphology). Vol. 6. Academic Press, New York and London: 1-79.
Hackenbrock, C. R. 1968 – Chemical and physical fixation of isolated mitochondria in low-energy and high-energy states. Proc. natn. Acad. Sci. U.S.A. 61: 598-605.
Haldar, D., Freeman, K. and Work, T. S. 1966 – Biogenesis of mitochondria. Nature 211(5044): 9-12.
Hall, J. B. 1973 – The nature of the host in the origin of the eukaryote cell. J. theor. Biol. 38: 413-418.
Hinde, R. and Smith, D. C. 1972 – Persistence of functional chloroplasts in *Elysia viridis* (Opisthobranchia, Sacoglossa). Nature New Biology 239: 30-31.
Holm-Hansen, O., Taylor, F. J. R. and Barsdate, R. J. 1970 – A ciliate red tide at Barrow, Alaska. Marine Biology, 7(1): 37-46.
Hovasse, R. 1965 – La détente des trichocystes des Cryptomonadines rendue explicable grâce à l'étude des particules (x) des *Paramecium aurelia* de race "Killer". C.R. Acad. Sci. 261: 2947-2949.

Hovasse, R., Mignot, J.-P. and Joyou, L. 1967 – Nouvelles observations sur les trichocystes des Cryptomonadines et les "R bodies" des particules kappa de *Paramecium aurelia*. Protistologica, 3(3): 241-256.
Hughes, D. E., Lloyd, D. and Brightwell, R. 1970 – Structure, function and distribution of organelles in prokaryotic and eukaryotic microbes. *In:* Charles, H. P. and Knight, B. D. [eds.] Organization and Control in Prokaryotic and Eukaryotic Cells, Vol. 20. 20th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge, England: 295-322.
Hustedt, F. 1930 – Die Kieselalgen Deutschlands, Osterreichs und der Schweiz, 1. *In:* Rabenhorst, L. Kryptogamenflora 7. Akademische Verlags.. Leipzig.
Hyman, L. H. 1940 – The Invertebrates: Protozoa through Ctenophora. McGraw-Hill Book Company, Inc., New York.
Jeon, K. W. 1972 – Development of cellular dependence on infective organisms: micrurgical studies in Amoebas. Science 176: 1122-1123.
Kalnins, V. I. and Porter, K. R. 1969 – Centriole replication during ciliogenesis in the chick tracheal epithelium. Z. Zellforsch. Mikrosk. Anat. 100: 1-29.
Kawaguti, S. and Yamasu, T. 1965 – Electron microscopy on the symbiosis between an elysoid gastropod and the chloroplasts of a green alga. Biol. J. Okayama Univ. 3: 57-65.
Kimura, M. and Ohta, T. 1973 – Eukaryote-prokaryote divergence estimated by 5S ribosomal RNA sequences. Nature New Biol. 243: 199-200.
Klein, R. and Cronquist, A. 1967 – A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphological and physiological characters in the Thallophytes. Quart. Rev. Biol. 42: 105-296.
Kubai, D. F. and Ris, H. 1969 – Division in the dinoflagellate *Gyrodinium cohnii* (Schiller). A new type of nuclear reproduction. J. Cell Biol. 40: 508-528.
Küntzel, H. 1969 – Proteins of mitochondrial and cytoplasmic ribosomes from *Neurospora crassa*. Nature (London) 222: 142-146.
Leadbeater, B. and Dodge, J. D. 1967 – An electron microscope study of nuclear and cell division in a dinoflagellate. Arch. Mikrobiol. 57: 239-254.
Lee, R. E. 1972 – Origin of plastids and the phylogeny of algae. Nature 237: 44-46.
Leedale, G. F. 1967 – Euglenoid Flagellates. Prentice Hall, Inc., Englewood Cliffs, New Jersey.
Ledoux, L. and Huart, R. 1971 – Fate of exogenous DNA in *Arabidopsis thaliana:* translocation and integration. Eur. J. Biochem. 23: 96-108.
Lewontin, R. C. 1972 – Testing the theory of natural selection. Nature 236: 181-182.
Luft, J. H. 1971 – Ultrastructure and function with regard to the origin of cell organelles: trials and tribulations. *In:* Harris, P. J. [ed.] Biological Ultrastructure: The Origin of Cell Organelles. Oregon State University Press, Corvallis: 1-23.
Lwoff, A. 1957 – The concept of virus. J. Gen. Microbiol. 17(1): 239-253.
McDonald, K. 1972 – The ultrastructure of mitosis in the marine red alga *Membranoptera platyphylla*. J. Phycol. 8: 156-166.
McLaughlin, P. J. and Dayhoff, M. O. 1973 – Eukaryotic evolution: a view based on cytochrome *c* sequence data. J. molec. Evolution 2: 99-116.
Manton, I. 1965 – Some phyletic implications of flagellar structure in plants. *In:* Preston, R. D. [ed.] Advances in Botanical Research, Vol. 2. Academic Press, London and New York: 1-34.
Margulis, L. 1970 – Origin of Eukaryotic Cells. New Haven and London, Yale University Press.
Mereschkowsky, C. 1905 – Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol. Centr. 25(18): 593-604.
Mereschkowsky, C. 1910 – Theorie der Zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. Biol. Centr. 30: 278, 321, 353.
Mereschkowsky, C. 1920 – La plante considérée comme un complexe symbiotique. Bull. soc. sci. nat. Ouest France 6(3): 17-21.
Meyer, R. R. 1973 – On the evolutionary origin of mitochondrial DNA. J. theor. Biol. 38: 647-663.

MIGNOT, J.-P., HOVASSE, R. and JOYON, L. 1970 – Nouvelles donnés sur le fonctionnement des trichocystes (= taeniobolocystes) des Cryptomonadines. J. Microscopie 9(1): 127-132.
MINCHIN, E. A. 1912 – An Introduction to the Study of the Protozoa. Arnold, London.
MORRÉ, D. J., MERRITT, W. D. and LEMBI, D. A. 1971 – Connections between mitochondria and endoplasmic reticulum in rat liver and onion stem. Protoplasma 73:43–49.
NASS, M. M. K. 1969a – Uptake of isolated chloroplasts by mammalian cells. Science 165: 1128-1131.
NASS, M. M. K. 1969b – Mitochondrial DNA: advances, problems and goals. Science 165: 25-35.
NASS, M. M. K. 1971 – Properties and biological significance of mitochondrial DNA. *In:* Harris, P. J. (ed.) Biological Ultrastructure: The Origin of Cell Organelles. Oregon State University Press, Corvallis: 41-63.
NASS, M. and NASS, S. 1963 – Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. J. Cell Biol. 19: 593–611.
NASS, S. 1969 – The significance of the structural and functional similarities of bacteria and mitochondria. Int. Rev. Cytol. 25: 55-129.
NASS, S. and NASS, M. 1963 – Intramitochondrial fibers with DNA characteristics. II. Enzymatic and other hydrolytic treatments. J. Cell Biol. 19: 613–629.
NICOL, J. A. C. 1960 – The Biology of Marine Animals. London, Pitman and Sons: 707 pp.
NORRIS, R. E. 1967 – Algal consortisms in marine plankton. *In:* Krishnamurthy, V. [ed.] Proc. Seminar on Sea, Salt and Plants: 178-189.
OAKLEY, B. R. and DODGE, J. D. 1973 – Mitosis in the Cryptophyceae. Nature 244: 521-522.
OLIVIERA, L. and BISALPUTRA, T. 1973 – Studies in the brown alga *Ectocarpus* in culture. I. General ultrastructure of the sporophytic vegetative cells. J. Submicr. Cytol. 5: 107-120.
PARSONS, D. F., WILLIAMS, G. R., THOMPSON, W., WILSON, D. and CHANCE, B. 1967 – Improvements in the procedure for purification of outer and inner membrane. *In:* Quagliariello, E., Papa, S., Slater, E. C. and Tager, T. M. (eds.) Mitochondrial Structure and Compartmentation. Bari, Adriatica Editrice: 29–73.
PARSONS, T. R. and BLACKBOURN, D. J. 1968 – Pigments of the ciliate *Mesodinium rubrum* (Lohmann). Netherlands J. Sea Res. 4(1): 27-31.
PASCHER, A. 1914 – Über Symbiosen von Spaltpilzen und Flagellaten mit Blaualgen. Ber. dtsch. botan. Ges. 32: 339-352.
PASCHER, A. 1929 – Studien über Symbiosen. I. Über einige Endosymbiosen von Blaualgen in Einzellern. Jahrb. Wiss. Bot. 71: 386-462.
PICKETT-HEAPS, J. D. 1969 – The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. Cytobios 3: 257–280.
PICKETT-HEAPS, J. D. and MARCHANT, H. J. 1972 – The phylogeny of the green algae: a new proposal. Cytobios 6: 255-262.
PIGOTT, G. H. and CARR, N. G. 1972 – Homology between nucleic acids of blue-green algae and chloroplasts of *Euglena gracilis*. Science 175: 1259–1261.
PREER, L. B., JURAND, A., PREER, J. R. Jr. and RUDMAN, B. M. 1972 – The classes of kappa in *Paramecium aurelia*. J. Cell Sci. 11: 581-600.
PRINGSHEIM, E. G. 1958 – Organismen mit blaugrünen Assimilatoren. Studies in Plant Physiology (Praha): 165–184.
DE PUYTORAC, P. and GRAIN, J. 1972 – Bacteriés intramitochondriales et particularités de l'ultrastructure cytostomo-pharyngienne chez le Cilié *Urotricha ovata* Kahl. C. R. Soc. Biol. 166(4-5): 604-607.
RAFF, R. A. and MAHLER, H. R. 1972 – The non symbiotic origin of mitochondria. Science 177: 575-582.
RAFF, R. A. and MAHLER, H. R. 1973 – Reply to Uzzell's and Spolsky's article, "Origin of Mitochondria". Science 180: 516-517.
RAIKOV, I. B. 1969 – The macronucleus of ciliates. *In*: Chen, T.T. [ed.] Research in Protozoology, Vol. 3. Pergamon Press, Oxford, London, New York: 1-128.
RANDALL, J. and DISBREY, C. 1965 – Evidence for the presence of DNA basal body sites in *Tetrahymena pyriformis*. Proc. roy. Soc. (Lond.) B 162: 473-491.

RAVEN, P. H. 1970 – A multiple origin for plastids and mitochondria. Science 169: 641-646.
RIS, H. 1961 – Ultrastructure and molecular organization of genetic systems. Canad. J. genet. Cytol. 3: 95-120.
RIS, H. and PLAUT, W. 1962 – Ultrastructure of DNA-containing areas in the chloroplast of *Chlamydomonas*. J. Cell Biol. 12: 383-391.
ROBERTSON, J. D. 1962 – The membrane of the living cell. Sci. Amer. 151: 3-10.
ROBERTSON, J. D. 1964 – Unit membranes: a review with recent new studies of experimental alterations and a new subunit structure in synaptic membranes. *In:* Locke, M. [ed.] Cellular Membranes in Development. 22nd Symposium of the Society for the Study of Development and Growth. Academic Press, New York and London: 1-81.
ROODYN, D. B. and WILKIE, D. 1968 – The Biogenesis of Mitochondria. London, Methuen & Co. Ltd.
RYTHER, J. H. 1967 – Occurrence of red water off Peru. Nature (London) 214: 1318-1319.
SAGAN, L. 1967 – The origin of mitosing cells. J. theor. Biol. 14: 225–274.
SCHIFF, J. A. and EPSTEIN, H. T. 1965 – The continuity of the chloroplast in *Euglena*. *In:* Locke, M. [ed.] Reproduction: Molecular, Subcellular and Cellular. Academic Press, New York-London: 131-189.
SCHIMPER, A. F. W. 1883 – Über die Entwicklung der Chlorophyllkorner und Färbkorner (1. Teil). Bot. Z. 41: 105–114.
SCHNEPF, E. 1966 – Organellen-Reduplikation und Zellkompartimentierung. *In:* Sitte, P. [ed.] Funktionelle und morphologische Organisation der Zelle. 3. wiss. Konf. Ges. dtsch. Naturf. u. Arzte: Probleme der biologischen Reduplikation. Springer, Berlin, Heidelberg, New York: 372-393.
SCHNEPF, E. and BROWN, R. M. 1971 – On relationships between endosymbiosis and the origin of plastids and mitochondria. *In:* Reinert, J. and Ursprung, H. [ed.] Origin and Continuity of Cell Organelles. (Results and Problems in Cell Differentiation Ser., Vol. 2). Springer-Verlag, Heidelberg: 299-322.
SCHUSTER, F. L. 1968 – The gullet and trichocysts of *Cyathomonas truncata*. Exper. Cell. Res. 49: 277-284.
SMITH-SONNEBORN, J. and PLAUT, W. 1967 – Evidence for the presence of DNA in the pellicle of *Paramecium*. J. Cell Sci. 2: 255-334.
SONEA, S. 1972 – Bacterial plasmids instrumental in the origin of eukaryotes? Rev. Can. Biol. 31(1): 61-63.
SONNEBORN, T. M. 1970 – Gene action in development. Proc. Roy. Soc. Lond. B. 176: 347-366.
STANIER, R. Y. 1970 – Some aspects of the biology of cells and their possible evolutionary significance. *In:* Charles, H. P. and Knight, B. D. [eds.] Organization and Control in Prokaryotic and Eukaryotic Cells, Vol. 20. 20th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge, England: 1–38.
STANIER, R. Y. and VAN NIEL, C. B. 1962 – The concept of a bacterium. Arch. Mikrobiol. 42: 17-35.
SWANSON, C. P. 1969 – The Cell. 3rd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey: 150 pp.
TAYLOR, D. L. 1967 – The occurrence and significance of endosymbiotic chloroplasts in the digestive glands of herbivorous Opisthobranchs. J. Phycol. 3: 234–235.
TAYLOR, D. L. 1968 – Chloroplasts as symbiotic organelles in the digestive gland of *Elysia viridis* (Gastropoda: Opisthobranchia). J. Mar. Biol. Ass. U.K. 48: 1-15.
TAYLOR, D. L. 1969 – Identity of zooxanthellae isolated from some Pacific Tridacnidae. J. Phycol. 5: 336-340.
TAYLOR, D. L. 1970 – Chloroplasts as symbiotic organelles. Int. Rev. Cytology 27: 29-64.
TAYLOR, D. L. 1971 – Photosynthesis of symbiotic chloroplasts in *Tridachia crispata* (Bërgh). Comp. Biochem. Physiol. 38A: 233–236.
TAYLOR, F. J. R., BLACKBOURN, D. J. and BLACKBOURN, J. 1969 – Ultrastructure of the chloroplasts and associated structures within the marine ciliate *Mesodinium rubrum* (Lohmann). Nature 224 (5221): 819-821.
TAYLOR, F. J. R., BLACKBOURN, D. J. and BLACKBOURN, J. 1971 – The redwater ciliate *Mesodinium rubrum* and its "incomplete symbionts": a review including new

ultrastructural observations. J. Fish. Res. Bd. Canada 28: 391-407.
TEWARI, J. P., MALHOTRA, S. K. and TU, J. C. 1973 – Ultrastructural transformation as a function of conformational states studies in freeze-fractures of rat liver mitochondria. Cytobios 7(25): 15-33.
TOMAS, R. N. and COX, E. R. 1973 – Observations on the symbiosis of *Peridinium balticum* and its intracellular alga. I. Ultrastructure. J. Phycol. 9(3): 304-323.
TOMAS, R. N., COX, E. R. and STEIDINGER, K. A. 1973 – *Peridinium balticum* (Levander) Lemmermann, an unusual dinoflagellate with a mesocaryotic and an eucaryotic nucleus. J. Phycol. 9(1): 91–98.
TRENCH, R. K. 1969 – Chloroplasts as functional endosymbionts in the mollusc *Tridachia crispata* (Bergh) (Opisthobranchia, Sacoglossa). Nature (London) 222: 1071-1072.
TRENCH, R. K., GREENE, R. W., and BYSTROM, B. G. 1969 – Chloroplasts as functional organelles in animal tissues. J. Cell Biol. 42: 404–417.
UZZELL, T. and SPOLSKY, C. 1973 – Origin of mitochondria. Science 180: 516-517.
WAGNER, R. P. 1969 – Genetics and phenogenetics of mitochondria. Science 163: 1026-1031.
WALLIN, I. E. 1927 – Symbionticism and the Origin of Species. Baillière, Tindall & Cox, Covent Garden, London.
WHALEY, W. G., DAUWALDER, M. and KEPHART, J. E. 1971 – Assembly, continuity and exchanges in certain cytoplasmic membrane systems. *In:* Reinert, J. and Ursprung, H. [eds.] Origin and Continuity of Cell Organelles. (Results and Problems in Cell Differentiation Ser., Vol. 2). Springer-Verlag, Heidelberg: 1–45.
WHITTON, B. A., CARR, N. G. and CRAIG, I. W. 1971 – A comparison of the fine structure and nucleic acid biochemistry of chloroplasts and blue-green algae. Protoplasma 72: 325-357.
WILKIE, D. 1970 – Reproduction of mitochondria and chloroplasts. *In:* Charles, H. P. and Knight, B. D. [eds.] Organization and Control in Prokaryotic and Eukaryotic Cells, Vol. 20. 20th Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge, England: 381-399.
WOOLHOUSE, J. W. 1967 – In a review of "The Plastids" by J. T. O. Kirk and R. A. E. Tilney-Bassett. New Phytol. 66: 832-833.
YAMATAKA, S. and HAYASHI, R. 1970 – Electron microscopic studies on the mitochondria and intramitochondrial microorganisms of *Halteria Geleiana*. J. electr. Microsc. 19(1): 50-62.
YOUNGER, K. B., BANERJEE, S., KELLEHER, J. K., WINSTON, M. and MARGULIS, L. 1972 – Evidence that the synchronized production of new basal bodies is not associated with DNA synthesis in *Stentor coeruleus*. J. Cell Sci. 11: 621–637.